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**USE OF ANTI-IDIOTYPES AND SYNTHETIC PEPTIDES FOR CONTROL OF  
HUMAN T-LYMPHOTROPIC VIRUS TYPE III INFECTIONS**

**ANNUAL PROGRESS REPORT**

by

Ronald C. Kennedy, Ph.D.

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) During the past year, we have continued our investigation on the potential use of synthetic peptides and anti-idiotypes (anti-Id) for controlling HIV infection. Previous studies have indicated that a peptide corresponding to amino acid sequences 735 to 752 from gp160 induced neutralizing antibodies in rabbits. Mouse monoclonal antibodies were generated to this peptide. These antibodies inhibited cell fusion of divergent HIV-1 isolates but failed to inhibit HIV-2. No neutralization by these monoclonal antibodies was observed in a VSV (HIV-1) pseudotype assay. These data suggest that this region of HIV gp41 may be involved in cell fusion of HIV infected cells with uninfected CD4 positive cells, but not in inhibiting the gp120/CD4 interaction.  We have also identified two gp41 synthetic peptides that, when coupled to KLH, exert a profound suppression of normal human proliferative responses to mitogens and alloantigens.					
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→ These peptides also inhibit normal human NK cell activity in vitro. Similar suppressive effects have been reported previously with a synthetic peptide analogous to amino acid sequences from the feline leukemia virus transmembrane glycoprotein.

→ Mouse monoclonal anti-Ids were generated against an affinity purified chimpanzee anti-gp41 preparation. This anti-Id was serologically characterized as representing a noninternal image Ab-2 preparation. Immunization of BALB/c mice with the anti-Id induced an Ab-3 response which bound HIV gp41 and expressed an Id that is shared with the chimpanzee Ab-1. The anti-Id induced anti-gp41 expressed a silent idiotype which was not expressed when BALB/c mice were immunized with a recombinant gp160. Rabbits were also immunized with this monoclonal anti-Id preparation. The anti-Id induced an Ab-3 response that was Id-positive, Ag-negative. The Id expressed on the rabbit Ab-3 is shared by the chimpanzee Ab-1. These data suggest that noninternal image anti-Id preparations can alter the serological characteristics of the immune response to gp160 in mice and rabbits.

→ Keywords: Vaccines Acquired Immune Deficiency Syndrome (AIDS) \*

Foreword:

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).



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#### A. Immunogenicity of HIV-1 env Synthetic Peptides

Monoclonal antibodies were generated against synthetic peptides analogous to amino acid sequences 735-752. Three IgM monoclonal antibodies were found to neutralize different HIV-1 isolates but not HIV-2 isolates. These antibodies failed to neutralize HIV-1 in a VSV pseudotype assay. The effect of these antibodies appears to be at a post binding step and may identify cell fusion epitopes on gp41.

In addition, we have synthesized a synthetic peptide that contains two contiguous epitopes, the 502-526 and 735-752 sequences. This dipeptide contains two B-cell epitopes which have been previously defined by our laboratories as inducing neutralizing antibodies in rabbits. Rabbits were immunized with this preparation in order to determine if we get an additive effect as it relates to neutralizing activity, when compared to rabbit antiserum produced upon immunization with synthetic peptides that represent the individual B-cell epitopes (either 503-532 or 735-752). Preliminary studies suggest that the antibody response in rabbit recognized primarily the neutralizing epitope associated with amino acid sequences 735 to 752. These studies will be helpful in understanding the concepts of a synthetic peptide based vaccine for HIV which contains several neutralizing B-cell determinants.

#### B. HIV-induced Immunosuppression by gp41 Synthetic Peptide

We have recently demonstrated a pronounced suppression of mitogen-induced blastogenic response in vitro by two synthetic peptides homologous to HIV gp160 amino acid sequences 735-752 and 846-860. Peptides conjugated to protein carriers exerted a profound suppression of the normal human lymphocyte proliferative response to ConA, PHA, PWM, and alloantigens. A synthetic peptide corresponding to a 17 amino acid sequence of the HIV TAT<sub>III</sub> gene product had no suppressive effects. The mechanism of immunosuppression remains unclear; however, our data suggest that suppression occurs at the level of IL-2 T cell interaction and that a down regulation of both IL-2 production and responsiveness may occur in HIV-peptide treated normal peripheral blood mononuclear cells.

We have also examined the ability of peptides 735-752 and 846-860, respectively, to inhibit normal natural killer (NK) cell activity in vitro. The two peptides exerted a significant inhibition on the normal NK cell activity as assessed against K562 tumor target cells in an in vitro radio-labeled Cr-release assay. This suppression of NK cell activity was observed only when the synthetic peptides were coupled to carrier proteins. Similarly coupled control peptides exhibited no suppressive effects. Addition of exogenous recombinant human interleukin-2 (IL-2) resulted in a partial restoration of the suppression of NK activity exerted by both peptides. Binding experiments indicated that peptides 735-752 and 846-860 did not affect the formation of effector cell-target cell conjugates. This suggests that one potential mechanism of the observed NK suppression is the inhibitory effect(s) subsequent to the formation of the lytic complex. These results suggest that the two peptides corresponding to sequences within the HIV transmembrane gp41 may play an important role in the pathogenesis of the defective NK cell activity in patients with AIDS.

### C. Anti-idiotypes Induce an Anti-HIV Response

A monoclonal anti-idiotypic antibody (anti-Id) was generated and characterized against a chimpanzee anti-gp41 preparation specific for synthetic peptide 735-752. This monoclonal anti-Id appeared to represent a noninternal image subclass of anti-Id. The anti-Id recognized the homologous chimpanzee anti-gp41 preparation, along with a second heterologous chimpanzee anti-gp41 induced by immunization with the HIV gp41 synthetic peptide. This anti-Id failed to recognize an interspecies Id expressed on rabbit and mouse monoclonal anti-gp41 preparations similarly induced by immunizations with peptide 735-752. Attesting further to the noninternal image nature of the anti-Id was its inability to inhibit the chimpanzee Ab-1 binding to peptide 735-752.

BALB/c mice and rabbits were immunized with this monoclonal anti-Id preparation. Mice produced an Ab-3 response that by ELISA recognized peptide 735-752 and a recombinant gp160 preparation. This murine Ab-3 response also recognized gp41 by Western blot analysis and inhibited the Id-anti-Id reaction. Thus, anti-Id immunization of mice induces an antigen (Ag) positive, Id positive response. The Id expressed on the murine Ab-3 response is not expressed in the anti-gp41 response of BALB/c mice immunized with peptide 735-752 (nominal antigen). The possibility exists that silent clones (Ag positive, Id positive) may have been activated within BALB/c mice immunized with this anti-Id. Rabbits immunized with the anti-Id produced an Ab-3 response that failed to bind either peptide 735-752 or HIV antigens. However, the rabbit Ab-3 inhibited the chimpanzee Ab-1-anti-Id reaction. This Ag negative, Id positive response induced in rabbits by anti-Id immunization shared an Id expressed on a chimpanzee anti-gp41 preparation. This Id is also not expressed in the rabbit anti-gp41 response induced by peptide 735-752.

Based on the above data, it was concluded that the monoclonal anti-Id did not exhibit any internal image activity and appeared to recognize a common nonantigen combining site Id present on two chimpanzee anti-gp41 preparations. The anti-Id failed to induce an anti-HIV response across species barriers. Failure of this anti-Id to induce an anti-HIV response across species barriers suggests its poor potential as a vaccine candidate. However, the rabbit Ab-3 expressed an Id that was similar to that expressed by the chimpanzee Ab-1. Within a given host it appears that noninternal image anti-Id may have the capacity to preprogram the Id response. The selection of the particular Id to be expressed during the Ab-3 response is based on the Id of the Ab-1 recognized by the noninternal image anti-Id. It may be important to identify those Ids induced during the immune response to HIV which are responsible for protective immunity. One can then utilize noninternal image anti-Id to select for an Id response that may preprogram a given host to induce a protective immune response when exposed to HIV.

Publications during the Past Year that Acknowledge Support of This Contract

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